

DOSE RESPONSE TRIAL IN MARGINAL HYPOPHOSPHATEMIA OF BUFFALOES

Benison Paul M., C.S. Randhawa, S. Chhabra* and Ashwani Kumar**ABSTRACT**

A dose response trial with oral phosphorus supplementation was conducted in hypophosphatemic (Plasma Pi 4.34 ± 0.24 mg/dl) buffaloes (n=14) by feeding dicalcium phosphate (DCP) daily for 2 months. All the animals under the study were lactationally and physiologically comparable. Blood, milk and rumen liquor samples were collected before, at 30th and 60th days of supplementation. Phosphorus supplementation improved body condition score (BCS) without any remarkable effect on plasma inorganic phosphorus (Pi), Calcium and ALP activity. No significant effect of oral P supplementation was observed on milk yield of buffaloes. Mean TVFA and ammonia nitrogen concentration in rumen fluid and plasma urea nitrogen and milk urea nitrogen were unaffected. The improved body condition in supplemented animals may be attributed to improved P responsive metabolism.

Keywords: *Bubalus bubalis*, buffaloes, body condition score, dicalcium phosphate, milk yield, plasma inorganic phosphorus

INTRODUCTION

Phosphorus has more known biological functions than any other mineral element. It is essential for broad range of enzymatic reactions particularly concerned with energy metabolism, energy transfer, transfer of genetic information, maintenance of cell membrane structure. Phosphorus is further involved in the control of appetite, in a manner not yet fully understood (Ternouth, 1990). Animals fed diet low in phosphorus were likely to suffer from poor appetite, pica and skeletal abnormalities (Suttle, 2010). Single sample baseline surveys conducted in dairy animals of Punjab during past years have shown the prevalence rate of hypophosphataemia, in 21.7% to 26.7% of buffaloes (Randhawa *et al.*, 2009; Singh 2002). Clinical observations however showed that many hypophosphatemic buffaloes do not show frank classical clinical signs. Thus, a differentiation has to be made whether hypophosphataemia is clinical, subclinical or acinical. Definite diagnosis of phosphorus deficiency is difficult. Although plasma phosphorus levels decline during phosphorus deficiency but its diagnostic value is limited because of lacking of tight homeostatic control mechanism. Plasma inorganic phosphorus concentration of 4.0 to 4.5 mg/dl is marginal for

ruminants. There are contrasting reports that the values of this order have been recorded in healthy grazing animals which do not respond to phosphorus supplementation (Coates and Ternouth, 1992) whereas other studies have shown positive response at higher blood levels. The present study was aimed to assess the effect of phosphorus supplementation on blood phosphorus status, physical performance, biochemical parameters and milk production in marginally hypophosphatemic buffaloes.

MATERIALS AND METHODS

The dose response trial was conducted in 14 buffaloes manifesting hypophosphataemia. These animals did not receive any mineral supplementation during last one year. Dicalcium phosphate (80 g) was fed daily for 2 months. All the animals under the study were lactationally and physiologically comparable.

Samples of blood, milk and rumen liquor were collected on 0, 30th and 60th days of feeding trial. Blood samples: (10 to 15 ml) were collected in heparinised mineral free glass vials. Plasma was harvested by centrifugation (3000 rpm for 15 minutes) and was stored at -10°C in deep freezer for subsequent analysis of inorganic phosphorus (Pi), Ca, ALP activity and plasma urea nitrogen (PUN). Plasma inorganic phosphorus (Pi) was estimated as per method of Taussky and Shorr (1953). Plasma Ca and PUN were analysed on RA-50 Autoanalyser using Autopak kit. Activity of ALP was assayed by method given by Bergemeyer (1974).

Rumen liquor

Samples were collected by per-cutaneous ruminal puncture with a 6 inch, 16 gauge needle.

Few drops (1 to 2) of saturated mercuric chloride solution was added to the rumen liquor, immediately after collection. The samples were sieved through a thin muslin cloth and stored at 4°C and analyzed for total volatile fatty acids (TVFA) and ammonia-nitrogen (NH₃-N).

Milk

Milk sample were collected in clean glass vials; 10 ml of milk was mixed with 10 ml of 24% TCA, kept in a shaker for 30 minutes and then filtered to obtain protein free- filtrate (PFF). The volume of PFF was measured and the clean PFF were analyzed for MUN.

Milk yield

Milk yield of two consecutive times (24 h) of the animals was recorded weekly.

Body Condition Score (BCS)

Both control and supplemented buffaloes were observed for body condition score at 0th, 30th and 60th days of the supplementation. BCS was measured on a scale of 1 to 5 given by Edmonson *et al.* (1989).

Seven hypophosphatemic buffaloes, being raised under similar feeding and management conditions were sampled as control group.

RESULTS AND DISCUSSION

Effects of oral P supplementation on plasma Pi, Ca and ALP activity of hypophosphatemic buffaloes:

Phosphorus supplementation did not alter the plasma Pi levels of hypophosphatemic animals as well as in control group throughout the experiment (Table 1). Similar to the present finding,

Brooks *et al.* (1984) supplemented about 39 cows with bone flour and monosodium acid phosphate on the evidence of hypophosphataemia (<1.30 mmol/l) which was not corrected in all the animals in spite of regular supplementation for 3 weeks. However, Karn (1997) observed variable effects on serum Pi values of supplemented and non-supplemented cows. In the first study significant improvement was noted in animals grazing on very low phosphorus pastures consistently for more than 9 months. However, in the second study either serum Pi of non-supplemented group showed higher values or non-significant differences from the supplemented group. In a long term (3 years) dose response trials by Espinoza *et al.* (1991), mean serum P was high in medium dietary phosphorus group (8%) than high (12%) and low (6%) during first year. However, a mean serum P was high in low dietary phosphorus group than medium and high groups during 2nd and 3rd years. Our results were also supported by the study of Wu *et al.* (2000) in lactating cows (milk yield > 10,000/L/ lactation) where plasma Pi declined during first 14 days of lactation with dietary phosphorus level of 0.03% compared to 0.40% and 0.49% and the difference leveled off during subsequent lactation period.

Contrary to the present finding, Sharma *et al.* (2002) recorded higher serum Pi, Ca and Mg following supplementation of a mineral mixture with 20% higher Ca, P, Mg for 75 days. The results of Brodison *et al.* (1989) also differed from the findings of present study. A consistently low blood P concentration was obtained in low P groups (4 to 4.5 g/kg DM), than high P group (6.0 to 6.5 g/kg DM) in all the 3 winter periods.

Valk *et al.* 2002 observed higher plasma Pi levels in high yielding cows (18 to 28 L/day) by increasing dietary P from 0.22 to 0.26% to 0.26 to 0.29%. The difference may be related to higher

lactation loss of phosphorus compared to the present study (milk yield 5 to 6 L/day).

The mean plasma Ca level on day 60 Post supplementation (9.49±0.21 mg/dl) was significantly higher than that of day 30 (Table 1). However, throughout the study plasma Ca levels of supplemented and non-supplemented group were statistically comparable at all samplings. Mean plasma ALP activity of both supplemented and non-supplemented animals were also unaffected throughout the dose response study (Table 1).

Plasma ALP activity in the buffaloes was unaffected during supplementation period. Plasma Ca levels were also in the normal range for the species. These observations indicated that the hypophosphataemia observed was not clinical, since an increased ALP activity is characteristic of a clinical form of the condition.

Physical performance

Mean body condition score (BCS) of the supplemented animals showed a progressive improvement throughout the study. Mean BCS for the supplemented group improved significantly ($P < 0.05$) at 30th and 60th days of supplementation (Table 1). Whereas, BCS for the non-supplemented group showed non-significant variation from day 0 through 30th and 60th days post supplementation (Table 1).

A remarkable improvement was noted in the BCS of one supplemented buffalo, wherein the pre phosphorus supplementation BCS value was 2.5 and it increased to a value of 3.75 after 60 days. Phosphorus supplementation of this buffalo did not evoke any milk yield response, but caused a remarkable increase in milk fat (by 3%). The effect of DCP supplementation on BCS in the present study was well supported by the findings of Shupe *et al.* (1988). Espinoza *et al.* 1991 also reported high

weight gain in high P group compared to medium and low P groups. Within one year of study, a significantly ($P < 0.01$) lower BCS was observed in cows fed low P diet than fed with adequate diet. In contrast, Moriera *et al.* (2009) found no significant differences in body weight or body condition score of low and high P in diet. Brodison *et al.* (1989) also found no consistent effect of P supplementation (6.0 to 6.5 g/kg) on condition score of lactating cows. Thus, P supplementation improved BCS without any remarkable effect on plasma Pi, Ca and ALP activity. From review of dose response trials, Suttle (2010) has also stated that definite evidence of phosphorus insufficiency comes from improvement in performance following P supplementation whereas biochemical indices provide only a rough estimate.

Milk yield

A non significant response in milk yield to phosphorus supplementation was observed. The mean milk yield for supplemented and non-supplemented groups showed a non significant gradual decline throughout the study period. The mean milk yield of supplemented and non-supplemented groups were statistically similar ($P < 0.05$) at all periods of sampling. Thus, no significant effect of oral P supplementation on milk yield of buffaloes was obtained in the present study. The lowered milk yield observed during the post treatment periods might be due to other variables like decreased fodder availability and physiological factors. These findings were supported by Brodison *et al.* (1989) who studied the effects of dietary P on lactating cattle by offering diets which differed only in P content. No consistent effect of high P concentrate (6.0 to 6.5 g/kg) was obtained on any of the variable *viz.* milk yield, milk composition and condition score. The findings of their study differed

from the present study in that a significant effect on BCS was obtained in the present study. However, the finding of Kincaid *et al.* (1981) differed from that of present study. A significant reduction in the milk yield was obtained by feeding diets containing 0.30% P and concluded that maximum milk yield could not be obtained with diets containing such a low P content. The differences in milk production response may be due to the difference in loss of P through milk in our buffaloes compared to exotic dairy cattle.

Plasma Urea Nitrogen (PUN) and Milk Urea Nitrogen (MUN)

Mean PUN concentration varied non-significantly during 0th, 30th and 60th days post treatment. An initial non-significant increase was observed after 30 days but later the value declined to the levels comparable to pre-supplementation concentration. A similar non-significant variation was also observed in the non-supplemented group of animals. Mean PUN concentration of the supplemented and non-supplemented group were not statistically different ($P < 0.05$) for all the three periods of sampling.

Mean MUN value revealed that concentration varied non-significant from the start, through 30th and 60th day of supplementation (Table 1). Similar non-significant variation was observed in the non-supplemented group. Mean MUN concentration of the supplemented and non-supplemented group were comparable for 0th and 30th days but showed a significant difference at 60 days post treatment. This may be due to the fact that mean MUN tended to be lower in non-supplemented group even prior to supplementation. Therefore, significant difference in MUN at 60 days post supplementation between supplemented and non-supplemented group assumes no significance.

The primary effect of hypophosphataemia in ruminants is a depression of appetite (Field *et al.*, 1975). Thus a reduced intake is expected to cause secondary negative effects on ruminal fermentation and protein status of the animals. When there is a dietary deficiency of protein, ruminal ammonia concentrations are relatively low and proportion of nitrogen recycled back into the rumen as urea is increased, so MUN is highly correlated with ruminal ammonia (Thornton 1970; Hammond 1983).

Moreover, there is a close relationship between blood urea N and milk urea N (Oltner and Wiktorsson, 1983; Oltner *et al.*, 1985). Thus, in a dietary P deficiency a diminished ruminal ammonia levels can be expected due to its effects on DM intake. Thus lower ruminal ammonia levels should in turn be reflected as lower MUN and PUN. However, in the current study no significant effect of P supplementation on PUN was obtained. Pre-phosphorus feeding values for PUN (12.49±1.46 mg/dl) in the supplemented animals suggested

Table 1. Effect of oral P supplementation on Pi, calcium and ALP activity and physical performance and biochemical parameters.

Parameter		Day 0	Day 30	Day 60
Plasma Pi (mg/dl)	S (n=14)	4.34±0.24 ^a	4.98±0.25 ^a	4.97±0.23 ^a
	US (n=7)	4.66±0.33 ^a	4.81±0.45 ^a	4.77±0.32 ^a
Plasma Ca (mg/dl)	S (n=14)	9.07±0.18 ^{ab}	8.62±0.20 ^a	9.49±0.21 ^{bc}
	US (n=7)	9.54±0.23 ^{ac}	9.24±0.21 ^{ac}	9.24±0.25 ^{ac}
Plasma ALP activity (IU/L)	S (n=14)	37.14±2.98 ^a	35.32±2.52 ^a	30.70±2.39 ^a
	US (n=7)	33.13±4.62 ^a	32.54±6.39 ^a	28.56±4.13 ^a
BCS (1 to 5)	S (n=14)	2.77±0.09 ^a	3.11±0.07 ^b	3.18±0.07 ^b
	US (n=7)	2.96±0.11 ^a	3.00±0.09 ^a	3.10±0.12 ^a
Milk yield (kg/day)	S (n=14)	4.43±0.55 ^a	3.98±0.49 ^a	3.23±0.45 ^a
	US (n=7)	3.57±0.97 ^a	2.89±0.76 ^a	2.28±0.66 ^a
PUN (mg/dl)	S (n=14)	12.49±1.46 ^a	13.41±1.68 ^a	12.41±0.95 ^a
	US (n=7)	9.79±1.33 ^a	9.36±1.32 ^a	8.99±0.91 ^a
MUN (mg/dl)	S (n=14)	10.54±1.05 ^a	9.91±1.13 ^a	11.08±0.77 ^a
	US (n=7)	7.04±0.90 ^{ab}	7.9±1.09 ^{ab}	7.93±0.74 ^b
TVFA (mEq/L)	S (n=14)	103.21±2.54 ^a	104.50±4.30 ^a	104.52±3.67 ^a
	US (n=7)	102.00±5.17 ^a	100.29±6.01 ^a	100.57±5.84 ^a
Ammonia nitrogen (mg/dl)	S (n=14)	7.63±0.86 ^a	7.61±0.51 ^a	7.88±0.46 ^a
	US (n=7)	6.43±0.47 ^a	7.58±1.01 ^a	6.90±0.40 ^a

Means with different superscripts in a row or a column for a parameter differ significantly (P<0.05).

S = Supplemented; US = Unsupplemented

that dietary N intake and/or utilization was better in these animals. The mean PUN values of the non-supplemented group were also not too low to believe that protein intake was inadequate.

Ruminal total volatile fatty acids and ammonia nitrogen

Mean TVFA concentration in rumen fluid were unaffected by P supplementation. Total volatile fatty acid concentration in rumen liquor was comparable in P supplementation. Total volatile fatty acid concentration in rumen liquor was comparable in P supplemented and non-supplemented groups prior to the treatment as well as during P supplementation period. Ruminal NH₃-N concentration also showed no significant changes to P supplementation. Ruminal ammonia concentration of both supplemented and non-supplemented groups were comparable at all the samplings.

The results of this study indicated that there were no appreciable effect on rumen fermentation with P supplementation and suggested that dietary phosphorus was adequate to support optimal rumen fermentation. This finding was also supported by Valk *et al.* (2002) where dry matter digestibility was similar with dietary P level of 0.22%, 0.26% and 0.32%. In contrast, Karn (2001) stated that P supplementation in P deficient bovines may cause increase in dry matter intake. The concentration of PUN and MUN were also unaffected by supplementation. Thus, the utilization of dietary constituents appeared to be unaffected in otherwise hypophosphatemic buffaloes. The improved body condition in supplemented animals may be attributed to improved P responsive metabolism.

It may be concluded that P supplementation improves physical performance without any remarkable effect on plasma Pi, Ca biochemical

parameters and production.

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